PTI-609: A Novel Analgesic that Binds Filamin A to Control Opioid Signaling

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Abstract: Binding a critical pentapeptide region on the scaffolding protein filamin A regulates signaling of mu opioid receptors so that their activation should not result in the opioid tolerance, dependence and addiction associated with current opioid painkillers. Additionally, we show that compounds that bind this site on filamin A reduce release of inflammatory cytokines. PTI-609 is a new chemical entity that binds filamin A with picomolar affinity and also activates opioid receptors via a novel binding domain. PTI-609 and analogs have similar analgesic efficacy to morphine by oral administration in mice, provide some anti-inflammatory activity in the rat collagen-induced arthritis model, and show no conditioned place preference at analgesic doses, suggesting no potential for abuse and addiction. PTI-609 was designed after discovering filamin A as the high-affinity target of naltrexone or naloxone. Combined with opiates, ultra-low-dose naltrexone or naloxone can enhance and prolong the analgesia of the opiate alone and prevent or attenuate opioid tolerance, dependence and addictive properties. We will review here the mechanism of action of ultra-low-dose naltrexone and naloxone, the discovery of filamin A as their high-affinity target, and the rationale as to why the current, dual-function new chemical entity should not only be easier to develop but also more consistently efficacious than opioids combined with ultra-low-dose naltrexone. This new class of compounds, as well as the concept, screening assay and pharmacophore model, are covered in a family of recent patent applications.

Keywords: Analgesia, conditioned place preference, cytokine, filamin A, inflammation, signaling.

BACKGROUND

Unraveling the Mystery of Ultra-Low-Dose Opioid Antagonists

The predecessor to PTI-609 and this novel class of analgesics was the combination of a classical opiate such as oxycodone or morphine with “ultra-low-dose” naloxone or naltrexone, two common opioid antagonists. Although no such combination has been approved for clinical use, extensive preclinical and some clinical data exist. Ultra-low-dose naloxone or naltrexone can enhance opioid analgesia and attenuate analgesic tolerance and dependence (Fig. (1)), with a mechanism long hypothesized as a blockade of excitatory signaling opioid receptors [1-4]. Ultra-low-dose naltrexone can also reverse hyperalgesia caused by acute, low-dose opioids to produce analgesia [5]. Additionally, ultra-low-dose naltrexone attenuates opioid reward or addictive properties in conditioned place preference [6] and self-administration and reinstatement paradigms [7]. This body of work started over 20 years ago, with the initial finding that while opioid agonists normally inhibit, or shorten, the action potential duration of dorsal root ganglion cells, lower doses of opioid agonists induce an opposite, excitatory effect: a prolongation of the action potential [8, 9]. Crain and Shen subsequently showed that ultra-low-dose naloxone blocked the action potential prolongation at low picomolar concentrations, and in vivo, ultra-low-dose naloxone prevented the antinociceptive tolerance caused by chronic morphine (10 mg/kg, s.c., twice daily for 7 days). Rats treated with morphine + naloxone (NLX) showed stable tail-flick latencies over the week of treatment, whereas tail-flick latencies of rats receiving morphine alone declined to a level not significantly different from NLX alone. * p<0.05 and ** p<0.01 for morphine + NLX versus morphine. BLINE = pre-drug baseline; Reprinted from [4], with permission from Elsevier.

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naloxone’s prevention of opioid tolerance and dependence was elucidated 10 years after the initial demonstration of *in vivo* effects. It was clear that ultra-low-dose naloxone blocked the MOR-Gs coupling associated with chronic opioid administration, while simultaneously enhancing levels of coupling to MOR’s native G proteins. Strikingly, our co-immunoprecipitation data showed that in spinal cord of rats cotreated with morphine plus ultra-low-dose naloxone, MOR-Gi/o coupling levels greatly surpassed those of opioid-naïve rats [4], perhaps mediating the enhanced analgesia seen acutely with these combinations. Despite this elucidation of mechanism of action of ultra-low-dose naloxone or naltrexone, their actual binding site in regulating MOR coupling was unknown.

**Finding the Target**

Because naloxone prevents MOR-Gs coupling at concentrations well below its affinity for MOR and by influencing the coupling behavior of MORs, we considered proteins that interact with MOR and MOR-associated G proteins as the most likely targets, particularly those able to interact with multiple MORs. We first examined proteins that co-immunoprecipitated with Go-coupled MOR following morphine activation. We identified a 300kDa protein co-immunoprecipitating with Go-coupled MOR as filamin A (FLNA, 3CNK.pdb) and then demonstrated that naloxone and naltrexone bind to FLNA with 4 pM affinity [14]. This high-affinity interaction certainly could mediate the effects of ultra-low doses of these two compounds.

Best known for cross-linking cytoplasmic actin into dynamic scaffolds to control cell motility, filamins are large cytoplasmic proteins increasingly found to regulate cell signaling by interacting with over 30 different receptors and signaling molecules [15, 16], including MOR [17]. We deduced the precise, pentapeptide binding site on FLNA by using overlapping peptides within the C-terminal, since C-terminal FLNA was earlier shown to interact with MOR using a yeast-two hybrid [17]. We demonstrated the functional significance of this high-affinity interaction by using peptide fragments containing the binding site to prevent naloxone from binding full-length FLNA in organotypic striatal slice cultures. Without addition of the peptide, FLNA interacts with ultra-low-dose naloxone and naltrexone, to prevent chronic morphine-induced MOR-Gs coupling, but in the presence of the peptide fragment, naloxone loses its ability to block the Gs coupling (Fig. (2)). We also showed that this MOR-Gs coupling happens acutely but transiently, perhaps mediating the rewarding or euphoric properties of opioids [18]. In that study, the cAMP second messenger was measured and followed the timecourse of the Gs coupling (Fig. (3)). Co-treatment with naloxone, naltrexone or the pentapeptide FLNA fragment all blocked the increased cAMP accumulation.

This high-affinity binding site in C-terminal FLNA therefore appears to underlie the paradoxical enhancement of opioid analgesia and prevention of analgesic tolerance and dependence by ultra-low-dose opioid antagonists. The high-affinity interaction of naloxone or naltrexone with FLNA likely prevents a critical MOR-FLNA interaction that otherwise increases the probability of MOR coupling to Gs instead of Gi/o proteins. In identifying the binding site though which ultra-low-dose naloxone or naltrexone prevents MOR-Gs coupling, our data also revealed an important regulation of MOR-G protein coupling by filamin A. Additionally, the discovery of a novel, non-opioid binding site of naloxone and naltrexone underlying their “ultra-low-dose” effects opens avenues to developing a novel class of analgesics without the difficulties and constraints of combining ultra-low-dose opioid antagonists with opioid agonists.

**Variables in Defining an “Ultra-Low” Dose**

Why was ultra-low-dose naltrexone or naloxone not consistently effective? The most significant variable, somewhat surprisingly, was the precise, ultra-low, dose. Ultra-low-dose naloxone and naltrexone effects do not follow a typical dose-response pattern, but usually span several log units in the pg/kg to ng/kg range. There is a clear threshold, however, where increasing dose of naltrexone or naloxone starts to disrupt their effects at ultra-low doses. Unfortunately this fine line seems to change with sex, strain, age, nociceptive versus neuropathic pain, and possibly many other variables.

An examination of the antinociceptive effects of various ratios of naltrexone:oxycodeone (1:10⁵, 1:10³, and 1:10⁵) combined with a range of oxycodeone doses (0.03-3mg/kg) in male Swiss Webster mice illustrates the wide range of effective doses of naltrexone and the threshold where analgesic enhancement diminishes [19] Fig. (4). Whereas all
naltrexone:oxycodone dose ratios examined enhanced the areas under the curve (AUCs) of tailflick latencies of the oxycodone doses, the strongest antinociceptive effects were obtained with the 3 and 300 pg/kg naltrexone doses, which appear to enhance the antinociceptive effects of 3 mg/kg oxycodone to a greater extent than a 30 ng/kg dose.

The optimal doses of naltrexone can vary with the opioid agonist it is combined with as well as the sex of the animal.
The prevention of tolerance and withdrawal-associated hyperalgesia by ultra-low-dose naltrexone was demonstrated at 0.3, 3, 300 and 3000ng/kg naltrexone when combined with 3mg/kg morphine [20] and at 1pg/kg or 1ng/kg naltrexone when combined with oxycodone [21]. The higher naltrexone doses used in these studies were used in males, suggesting males are less sensitive to ultra-low-dose naltrexone effects. Hence, although male rodents are generally more sensitive to opioid analgesia [22], they appear to be less sensitive to ultra-low-dose naltrexone. These findings likely contribute to the variations in optimal ultra-low-dose naltrexone dose ranges noted both with sex of the animal and the opioid used in combination.

In addition to sex differences, the effects of ultra-low-dose naltrexone on morphine antinociception and tolerance can also vary with rat strain [23]. Whereas the enhancement of morphine antinociception and attenuation of morphine tolerance was observed in both Sprague-Dawley and Long-Evans rats, naltrexone (0.1-100ng/kg) did not enhance morphine antinociception in Fisher 344 or Lewis rats, nor did 10 or 100ng/kg naltrexone significantly attenuate tolerance in these two strains. Given that pg/kg doses of naltrexone most effectively enhanced oxycodone antinociception in female mice [21], perhaps this even lower dose range might enhance antinociception in these two rat strains. Terner (2006) also reported strain-dependent sex differences. Another example of strain differences is the initial report that ultra-low-dose naltrexone enhances morphine antinociception in Swiss Webster mice but antagonizes it in 129/SvEv mice [24]. A final study in rats noted sex effects as well as age effects: morphine antinociception was enhanced by low-dose naltrexone in mature female but not mature male rats (18-22 weeks) and was negligible in younger rats [25]. However, the naltrexone dose range used in that study was again comparatively high (0.002-2μg/kg), and the effect in mature females was “inversely related to dose.”

Neuropathic pain is typically not well treated by opioids, but ultra-low-dose naltrexone can enhance their effects. Interestingly, the doses of naltrexone used to enhance the anti-hyperalgesic effects of oxycodone are notably higher than the dose ranges mentioned above for nociceptive pain. In the spinal nerve ligation model of neuropathic pain, naltrexone enhanced the effectiveness of oxycodone and greatly diminished the tolerance seen with repeated administrations [26]. Naltrexone was administered at 3μg/kg, a dose 10-to 100-fold higher than those used to enhance analgesia and alleviate tolerance in nociceptive pain.

In summary, all of these studies show that an effective ultra-low dose of naloxone or naltrexone seems to depend on many interacting variables, including sex, strain, age, the opioid agonist used in combination, and type of pain. These variables may reflect underlying differences in FLNA associations, conformations or content.

Clinical Experience with Ultra-Low-Dose Opioid Antagonists

The inconsistent effects seen in clinical studies may also be related to the actual dose of naltrexone or naloxone and the variables defining the too-high threshold dose. Clinical experience with opioid antagonists combined with opiates is limited to small clinical studies [27-31], and two clinical
trials [32, 33]. In a notable case study, a diabetic polyneuropathy patient, who previously had no pain relief from a variety of treatments, reported profound analgesia when 2μg/day of naltrexone was co-administered with methadone [28]. The first controlled clinical study demonstrated an opioid-sparing effect and a reduction in side effects by a continuous infusion of naloxone at 0.25μg/kg/hr added to morphine administered by patient controlled analgesia (PCA) [27]. Using nalmefone, an opioid antagonist not yet confirmed to bind filamin A, another study reported decreased severity of pain 24 hours later and a decreased need for antiemetics and antipruritics following a single 15 or 25μg injection prior to morphine PCA [31]. Cepeda and colleagues were unable to replicate these effects using a higher dose of naloxone mixed with morphine for PCA [29] and subsequently showed only a decrease in side effects using a much lower naloxone dose [30]. More recently, buprenorphine analgesia was significantly enhanced by naloxone at 2.3μg for a 70kg patient but not by higher and lower doses of naloxone [34].

Although it is difficult to compare doses of naloxone or naltrexone in these small clinical studies, it is clear that the analgesic-enhancing effect is not consistently seen, even if dose levels seem to be consistent. A clear naltrexone dose-effect relationship was noted, however, in two clinical trials of Oxytrex™ (oxycodone + ultra-low-dose naltrexone). The first showed a significant increase in analgesia over oxycodone alone if patients received 2 but not 4μg/day of naltrexone [32]. This study also showed a slight sex difference (Fig. (5)). In a phase III trial of Oxytrex, significant decreases in physical dependence as well as in constipation, somnolence and pruritis were observed in patients receiving 2 but not 4μg/day of naltrexone [33]. In this latter trial, patients were allowed to titrate their dose by taking higher (or lower) strength tablets to achieve sufficient analgesia, but with the naltrexone in Oxytrex tablets fixed at 1μg/tablet, patients received a consistent 2 or 4μg naltrexone/day, depending on bid or qid dose regimens. Interestingly, the total average daily dose after self-titration was 12% lower for both bid and qid Oxytrex groups compared to the qid group receiving oxycodone with no naltrexone.

Two Binding Sites on Filamin A

One wonders how it can be that 2 but not 4μg/day of naltrexone can enhance analgesia clinically, and how such a broad dose range below this threshold can be effective while crossing the line, as variable as it is, destroys the effects. Intuitively, the obvious thought was that the additional opioid antagonist starts to disrupt the opioid agonist, thereby diminishing the overall effect. However, a nanogram or microgram dose of opioid antagonist is not likely to noticeably disrupt the biological effects of a milligram dose of opioid agonist. Evidence that the possibility of spillover to opioid receptors is not the case comes from a study examining the reduction in rewarding properties of oxycodone by ultra-low-dose naltrexone [6]. In this study, 0.03 or 0.3ng/kg naltrexone blocked a conditioned place preference (CPP) to 3mg/kg oxycodone, whereas 3ng/kg naltrexone did not block this CPP to oxycodone (Fig. (6)). As the naltrexone dose increased to 30ng/kg, the CPP to oxycodone is not significant. The robust CPP in the middle of this dose-response curve demonstrates a point where the “ultra-low-dose effects” are lost and the classical antagonism of opioid receptors is not yet occurring. In analgesic paradigms, if opioid receptors are antagonized, analgesia would be decreased, just as it would when crossing the ultra-low-dose threshold. However, in the CPP paradigm, when the ultra-

![Fig. (5). Reduction in pain intensity in males and females by Oxytrex. Oxytrex BID, a daily dose of 2 μg naltrexone, provided the greatest reduction in pain intensity scores in both males and females. At Week 3, Oxytrex BID was significantly better than placebo in males and significantly better than oxycodone in females. The pain intensity scores of the Oxytrex QID group, receiving 4 μg naltrexone/day, did not statistically separate from placebo. Reprinted from [32] with permission from Elsevier.](image)
low-dose effect is lost, the CPP to oxycodone reappears; whereas, antagonizing opioid receptors would interfere with the CPP.

What then is disrupting the ultra-low-dose naloxone/naltrexone effects, making this threshold of efficacy such a fine line to tread? We believe this threshold is due to a second binding site of naloxone and naltrexone on the FLNA protein. A competition (displacement) curve for the inhibition of tritiated naloxone binding by naltrexone to membranes from FLNA-expressing cells shows two affinity states with IC50-high of 3.94 picomolar and IC50-low of 834 picomolar Fig. (7) [14]. It is very possible that binding the almost nanomolar binding site on FLNA may alter the conformation of the protein or disrupt some conformational change induced by binding the picomolar binding site. This two-hundred-fold difference in affinity could represent the fine line of whether ultra-low-dose naloxone or naltrexone enhances opioid analgesia, prevents tolerance and dependence and attenuates opioid reward or not.

A NEW APPROACH: PTI-609

Now that the high-affinity binding site of naloxone and naltrexone is known to be a pentapeptide region on FLNA, we were able to design novel compounds that combine high-affinity FLNA binding with MOR agonist activity in a single, new chemical entity. Our approach was molecular modeling, virtual screening of the world’s compounds against the model, followed by actual screening of a small number of selected compounds for binding to the FLNA pentapeptide and activation of MOR. The screening data was used to refine the model, and the cycle was repeated once more before initiating medicinal chemistry, which produced several good lead candidates with fM to pM affinity to the FLNA pentapeptide and good analgesic efficacy in mice by oral administration. The analgesic dose-response curve of orally administered PTI-609 indicates that PTI-609 is bioavailable and CNS penetrable Fig. (8). The initial patent application covered the concept, the screening assay and an early pharmacophore model [35], and several patent applications covering families of these novel compounds have also published [36-38]. These novel compounds do not structurally resemble known opioids, and interestingly, the three lead compounds that were tested do not displace diprenorphine in a binding assay, suggesting a novel binding domain in activating MOR. Functional activation of MOR in human post-mortem caudate tissue using a GTP\S binding assay shows agonist activity comparable to the full agonist [D-Ala2, N-MePhe4, Gly-ol]-enkephalin (DAMGO). At 0.1 μM concentration, stimulation of binding was 204.6 ± 52.6% for PTI-609 and 210.6 ± 34.0% for DAMGO.

The dual function of PTI-609 avoids the difficulties of developing a combination product, allowing easier formulation and simpler clinical trials. Most importantly, by screening for compounds that bind only the picomolar binding site on FLNA and not the second, lower affinity site on this scaffolding protein, we avoid the difficulties in treading the ever-moving, fine line of the ultra-low dose of naloxone or naltrexone. To confirm that this sand-trap is now a non-issue, we can simply increase concentrations of these compounds and confirm that effects are not lost (see Anti-inflammatory effects section). What is expected is an analgesic suited for moderate-to-severe pain with little to no
tolerance, dependence and addictive potential. Additionally, we anticipated some anti-inflammatory activity.

**Anti-Inflammatory Effects**

Why would we expect anti-inflammatory effects from compounds that bind this site on FLNA? The laboratory of Jau-Shoyng Hong has shown that both (+) and (-)-naloxone inhibit lipopolysaccharide (LPS)-induced activation of microglia and release of proinflammatory factors in mixed neuron-glial cultures at femtomolar concentrations [39, 40]. The authors describe this anti-inflammatory and neuroprotective response as bimodal, because the protection is lost in the high picomolar range before reappearing in the micromolar range. This threshold is reminiscent of the loss of effects of naloxone and naltrexone in potentiating opioid analgesia, and knowing that naltrexone binds FLNA in the femtomolar to picomolar range, we suspected that these

Fig. (7). Naloxone binds FLNA with picomolar affinity. A competition (displacement) curve for the inhibition of [3H]-naloxone (NLX) binding by naltrexone to membranes from FLNA-expressing A7 cells shows two affinity states with IC$_{50}$-H of 3.94 picomolar and IC$_{50}$-L of 834 picomolar. A nonlinear curve-fit analysis was performed using a competition equation that assumed two saturable sites for the naltrexone curve comprising of 16 concentrations ranging from 0.1pM to 1μM. Data are derived from 6 experiments each using a different set of A7 cells. Reprinted from [14].

Fig. (8). Analgesic efficacy of PTI-609 by oral administration in mice. Mice were tested in the 52°C hot water immersion test using a 10-s cutoff, which represents 100% maximal possible effect (MPE). Data are means ± SEM. n=6.
effects could be the result of FLNA binding. The Hong laboratory suggested NADPH oxidase as a target, which appears to be a critical final common pathway enzyme in producing both extracellular and intracellular reactive oxygen species, and subsequently, inflammatory cytokine release.

The Linda R. Watkins laboratory has also demonstrated that naloxone and naltrexone block glial activation and resulting proinflammatory cytokine release [41]. The target suggested by the Watkins laboratory in these effects is toll-like receptor 4 (TLR4) of innate immune cells, because naloxone and naltrexone, including their isomers that are inactive at opioid receptors, block TLR4 signaling [41, 42]. A large body of work, led by Dr. Watkins, has demonstrated the role of glial activation in the negative effects of opioids, including opioid tolerance, dependence, reward, and respiratory depression [43], so it is possible that ultra-low-dose opioid antagonists are alleviating these effects by diminishing the glial activation of opioids. Although the glial activation could be a downstream effect of MOR-Gs signaling, the cell line used to demonstrate that (+) and (-) naloxone disrupt TLR4 signaling was devoid of opioid receptors [42]. Hence, binding to FLNA in glial cells may slow down vesicular release of cytokines, or even disrupt TLR4 signaling. Although FLNA binders such as (+)naloxone have not been reported to have antinociceptive effects themselves, they can reduce allodynia in a model of neuropathic pain [40], and this effect is attributed to their suppression of glial activation.

We first assessed cytokine release from LPS-stimulated human astrocytes and the potential blockade of this cytokine release by PTI-609 and related novel compounds. We assessed concentrations from 100 fM to 100 nM and compared these to 10 pM and 1 nM of (+)naloxone. Whereas PTI-609 and two related compounds potently reduced release of IL-6, IL-1β and TNF-α, (+)naloxone suppressed release of these cytokines somewhat less potently at 10 pM and virtually not at all at 1 nM (Wang and Burns, unpublished data, Fig. (9)). This loss of efficacy by (+)naloxone at 1 nM again demonstrates the crossing of the ultra-low-dose threshold, likely mediated by binding to the second binding site on FLNA, the site it binds with 834 pM affinity. The lack of dose-response in this initial test of cytokine release suggests that naloxone and naltrexone’s picomolar binding site is already saturated by these com-pounds at 100 fM. Lower concentrations may show a true dose-response.

We next assessed anti-inflammatory activity of PTI-609 in the rat collagen-induced arthritis model. Animals were dosed twice daily by oral gavage beginning on the day of the second collagen injection. Three different dose groups of the novel compound were compared to 3mg/kg celecoxib and 25mg/kg ibuprofen. PTI-609 significantly reduced foot volume at 56mg/kg and at one time point at 5.6mg/kg, but not at 32mg/kg. The reduction in foot volume was far less potent than that produced by celecoxib, and approximately half that seen with ibuprofen in this initial proof of concept study (Wang and Burns, unpublished data, Fig. (10)).

Addictive Potential

Our data linking the immediate and transient MOR-Gs coupling to CREB activation, as well as the blockade of conditioned place preference (CPP) by pg/kg doses of naltrexone, suggested that our novel compounds would have reduced addictive potential. We previously showed that in brain slices, acute high dose morphine caused an immediate but transient Gs coupling by MOR that was associated with activation of cyclic AMP response element-binding protein (CREB) [18], a transcription factor implicated in addiction and withdrawal effects [44-48]. Blockade of this immediate MOR-Gs coupling by picomolar concentrations of naloxone or by its pentapeptide binding site on FLNA blocked the associated activation of CREB. The rewarding effects of opioid drugs are most pronounced as plasma levels are rising quickly and are less intense or even negative later [49-51], suggesting that this immediate Gs coupling is at least temporally associated with the most intense phase of opioid reward. To support the hypothesis that our FLNA-binding novel compounds would not have the same rewarding properties of classical opioids, we examined equi-analgesic doses of PTI-609 and oxycodone in the CPP paradigm. Oral doses that produced an 80-90% analgesic effect as well as a dose ¼ log lower were compared to a vehicle group, using three conditioning sessions to the drug-paired compartment as well as to the vehicle-paired compartment. A significant CPP to both doses of oxycodone was observed, whereas in contrast, there was no change from baseline in time spent in the putative conditioning side with either dose of PTI-609.
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This result suggests that at therapeutic doses, no rewarding effects occur, or at least none strong enough to produce a CPP. We attribute this lack of CPP to the FLNA-binding capacity of PTI-609 and its prevention of MOR-Gs coupling; however, the activation of MOR via a novel binding site, i.e. the compound’s inability to displace diprenorphine in a binding assay, may also contribute. Additionally, according to the work linking glial activation with opioid reward [43], the fact that these compounds potently suppress cytokine release by activated glia may also help prevent rewarding effects despite activation of MOR.

CURRENT & FUTURE DEVELOPMENTS

This review described the discovery of the FLNA target bound with picomolar affinity by naloxone and naltrexone, and the difficulties and inconsistencies with combining opioids with ultra-low-dose naloxone or naltrexone because they bind a second, lower-affinity binding site on this protein. While the development of such opioid agonist/antagonist combinations has essentially halted, a new direction has been initiated with PTI-609, a new chemical entity that, in a single molecule, binds the appropriate pentapeptide region of FLNA with high affinity and activates MOR via a novel binding site. Importantly, unlike naloxone or naltrexone, PTI-609 reduced foot swelling in female Wistar rats immunized with type-II collagen on days 1 and 9. PTI-609 was administered at 5.6, 32 and 56 mg/kg starting on day 9. Significant differences from vehicle (p<0.05) were achieved on Days 17, 20 and 23 with the high dose, and on Day 20 and 23 with the low dose of PTI-609.

Fig. (10). Reduction in foot swelling in rat collagen-induced arthritis model. Oral twice daily administration of PTI-609 reduced foot swelling in female Wistar rats immunized with type-II collagen on days 1 and 9. PTI-609 was administered at 5.6, 32 and 56 mg/kg starting on day 9. Significant differences from vehicle (p<0.05) were achieved on Days 17, 20 and 23 with the high dose, and on Day 20 and 23 with the low dose of PTI-609.

Fig. (11). Lack of conditioned place preference with PTI-609. In mice given three 20-minute conditioning sessions to oral administration of drug or vehicle versus saline, there was a significant place preference to both doses of oxycodone but no change in time spent in the putative conditioning compartment in the groups receiving PTI-609. Data are means ± SEM.
xone, PTI-609 avoids the second, lower-affinity binding site on FLNA and therefore should maintain its beneficial effects with increasing dose, allowing the picomolar site to saturate with no interference. Although much work remains before PTI-609 can enter clinical trials, the early research presented here shows PTI-609 and related compounds to be efficacious analgesics that possess additional anti-inflammatory properties and, according to the standard conditioned place preference paradigm, may not carry the abuse and addictive potential of classical opioid drugs. The problems with current opioid painkillers, from side effects to tolerance, dependence, abuse, diversion and addiction demonstrate a great need for a better class of efficacious analgesics. PTI-609 is a promising new drug candidate that seems well poised to fulfill that need.

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CONFLICT OF INTEREST

L.H. Burns is an employee of Pain Therapeutics Inc., the company that funded the development work on PTI-609 as well as earlier work on Oxytrecx.

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